

Phytochemical analysis and *in vitro* antibacterial activity of root peel extract of *Raphanus sativus* L. var niger

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ABSTRACT

The extracts of peels of edible root of *Raphanus sativus* L. var niger were analyzed for phytochemicals and *in vitro* antibacterial activity. The proximate analysis and phytochemical analysis revealed that peels of *R. sativus* L. var niger had most of the important phyto-constituents like tannins, saponins, flavonoids, phlobatannins, anthraquinones, carbohydrates, reducing sugars, steroids, phytosterol, alkaloids, amino acids, terpenoids, cardiac glycosides and chalcones; indicating its potential for medicinal use. Agar well diffusion assay was employed to test the antibacterial activity of extracts, prepared by using different solvents, against gram positive *Staphylococcus aureus* ATCC 12598, *Bacillus subtilis*-QAU and *Micrococcus luteus* ATCC 10240 and gram negative bacteria *Escherichia coli*-ATCC 8739, *Salmonella typhi*- ATCC 14079, *Klebsiella pneumonia*-QAU, *Pseudomonas aeruginosa* ATCC 7700, *Bordetella bronchiseptica* ATCC 4617 and *Enterobacter aerogenes*-QAU. Effectiveness of the extracts (CAE, CEE, CME, CEAE and CPEE) against different bacterial strains was measured in terms of zone of inhibition in millimeters. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values determined were compared with the positive control (Gentamycin) used. The present study is supportive evidence that peels of *R. sativus*, generally wasted, have important medicinal constituents.

Keywords: *Raphanus sativus* L. var niger, antibacterial activity, minimum inhibitory concentration, minimum bactericidal concentration.

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INTRODUCTION

Plants being the most reliable source of curatives are used as folk medicines for centuries. Even 80% of the modern day man still focuses on plant based remedies, for their better adaptability, lesser side effects and economical affordability with a huge compliment to the cultural acceptance (Firas and Bayati, 2009).

R. sativus niger belongs to family Brassicaceae (order Brassicales, subspecies niger and variety niger). It is a food crop, mostly an ingredient of salads in Asian countries during winter. Its familiar names include black radish (English), Mooli (Urdu) and Daikon (Japanese). It has been used as a medicinal plant from a long time. It has laxative effects on intestine and acts as an appetizer (Chevallier, 1996), used for curing liver dysfunction and poor digestion (Gutierrez and Perez, 2004; Lugasi et al., 2005), acts as antioxidant (Lugasi et al., 2005; Wang et

al., 2010), anti-tumorigenic (Kim et al., 2011), anti-mutagenic (Nakamura et al., 2008), anti-diabetic (Shukla et al., 2010), and anti-proliferative (Papi et al., 2008; Yamasaki et al., 2009; Beevi et al., 2009). It is also very well known for its use in the treatment of bronchitis and diarrhea (Bown, 1995; Chevallier, 1996).

Medicinal activities of plants have long been associated with the production of secondary metabolites which include tannins, terpenoides, coumarins, alkaloids and flavonoids. These products help plant to carry out various activities like defense and pollination. However, their antioxidant, antimicrobial and other medicinal properties are widely exploited for the benefit of mankind regarding healthcare. Certain biological assays are conducted in order to assess the phytochemicals and antimicrobial potentials of a plant (Cowan, 1999).

R. sativus is found to be effective against different bacterial strains including pathogenic bacteria: *Escherichia coli*, *Pseudomonas pyocyaneus*, *Salmonella typhi*, *Bacillus subtilis*, *Staphylococcus aureus*, *Streptococci*, *Pneumococci*, *Listeria*, *Micrococcus*, *Enterococcus*, *Lactobacillus* and *Pedococcus* (Abdou, 1972; Yeung, 1985; Rani et al., 2008; Shukla et al., 2011).

As compared to synthetic antimicrobial agents, plant based antimicrobials are cost effective, affordable and exhibit lesser side effects. As microbes are rapidly evolving their defense mechanism, so does the resistance develops against many of the antibiotics which were once effective. The search for new antimicrobial compounds has always been a need. The present study was designed to dig out the antibacterial potential of waste material like peels of *R. sativus*. This preliminary study support the fact that, like seeds (Rani et al., 2008), roots (Esaki and Onozaki, 1982) and leaves (Firas and Bayati, 2009) of *R. sativus*, peels also show antimicrobial activity against some pathogenic gram positive and gram negative bacteria.

MATERIALS AND METHODS

Sample collection

Root peels of *R. sativus*, a common vegetable used as a salad in winters, were collected from kitchen and allowed to dry in shade for 2 weeks. To prevent the loss of active phytoconstituents, samples were kept under constant observation to avoid any fungal growth.

Sample preparation

Dried peels were ground in an electric grinder to obtain fine powder. The powdered sample was stored in sterilized air tight container at room temperature (25-30°C).

Extract preparation

Extracts of *R. sativus* niger root peel sample were prepared using different types of organic solvents and water.

Aqueous extract

To obtain the crude aqueous extract (CAE), 1 g of powdered sample was mixed with 50 ml of sterilized distilled water. The mixture was incubated at 25°C with constant shaking at 150 rpm for three days (72 h) in orbital shaker (Techno OS-290). Extract was filtered using Whatman filter paper and filtrate was then allowed to evaporate at 40°C.

Methanolic extract

Eighty percent (80%) methanol was used to prepare methanolic extract of sample. In 50 ml solvent, 1 g of sample was added. After constant shaking at 150 rpm for 72 h at room temperature, the sample was filtered. Filtrate was incubated at 40°C till all the solvent was evaporated leaving behind the crude methanolic extract (CME).

Ethanollic extract

Ethanollic extract was prepared using 95% ethanol. In 50 ml 80% ethanol, 1 g of sample was added. After constant shaking at 150 rpm for 72 h at room temperature, the sample was filtered. Filtrate was incubated at 40°C till all the solvent was evaporated leaving behind the crude ethanollic extract (CEE).

Soxhlet Extracts

Soxhlet extractor was used for preparing ethyl acetate and petroleum ether (40 to 60°C) extract. 7 g of *R. sativus* niger peel powder was used for extraction with 150 ml of solvent including ethyl acetate and petroleum ether (40 to 60°C) separately were used for extraction at 50 to 60°C for 7 h (AOAC, 1995). Finally, the crude ethyl acetate extract (CEAE) and crude petroleum ether extracts (CPEE) are obtained.

Percent yield

The percentage yield of extract for different solvents was calculated using the formula:

$$\text{Percentage yield} = \frac{\text{Weight of final extract}}{\text{Weight of powdered sample}} \times 100$$

Phytochemical analysis

For qualitative analysis of active phytochemicals in *R. sativus* niger roots peel extract. Preliminary Phytochemical analysis were carried out on CAE, CME, CEE, CEAE and CPEE using standard protocol for determination of phytoconstituents including: tannins, saponins, phlobatannins, anthraquinones, carbohydrates, reducing sugars, steroids, phytosterol, flavonoids, alkaloids, amino acids, terpenoids, chalcones and cardiac glycosides as described by Trease and Evans (1978), Sofowora (1994), Harborne and Harborne (1998), Kokate (2001), Kaur and Arora (2009), and Kumar et al. (2011).

Proximate analysis

Moisture and dry content

Total moisture of the sample was determined according to AOAC (1995). Dry content was calculated by subtracting value of moisture content from 100.

Crude Protein

Kjeldahl's method (AOAC, 1995) was used to estimate nitrogen content and total crude protein using factor (6.25).

Crude fibre and ash content

Crude fibre content and ash content was determined by using methods of AOAC (1995).

Crude fats

Crude fats in the sample were determined by Soxhlet extraction method using n-hexane as solvent (AOAC, 1995).

Table 1. Phytochemical analysis of extracts of *Raphanus sativus niger*.

Phytochemicals	Solvents			Soxhlet extract	
	CAE	CME	CEE	CPEE	CEAE
Tannins	+	+	+	+	+
Saponins	-	+	+	+	+
Flavonoids	+	+	+	+	+
Phlobatannins	-	+	+	+	-
Anthraquinones	-	-	-	+	+
Carbohydrates	-	-	+	+	+
Reducing sugars	-	-	+	-	-
Steroids	-	-	+	+	+
Phytosterol	-	+	+	+	+
Alkaloids	+	+	+	+	+
Amino acids	-	+	-	+	+
Terpenoids	-	+	+	+	+
Cardiac glycosides	-	-	+	+	+
Chalcones	-	+	+	+	+

Total Carbohydrates

Total carbohydrates in the sample were estimated by finding the difference [100-(crude protein + crude fats + ash + crude fibre)] (Khalifa, 1996).

Determination of antibacterial activity

Bacterial strains and culturing

Six out of nine bacterial strains used in the study were ATCC pathogenic bacterial strains (*Staphylococcus aureus* ATCC 12598, *Micrococcus luteus* ATCC 10240, *Escherichia coli* ATCC 8739, *Salmonella typhi* ATCC 14079, *Pseudomonas aeruginosa* ATCC 7700 and *Bordetella bronchiseptica* ATCC 4617) and other three strains were obtained from Department of Biochemistry, Quaid e Azam University, Islamabad, Pakistan (*Bacillus subtilis*- QAU, *Klebsiella pneumoniae*-QAU and *Enterobacter aerogenes*-QAU). These strains were cultured at 37°C except *Micrococcus luteus*, which was grown at 25°C, and all were maintained at 4°C using LB media.

Agar Well Diffusion Assay

Agar well diffusion assay was performed to determine the activity against bacterial strains (Etebong and Nwafor, 2009). Lysogeny Broth (LB) agar plates were seeded with bacterial culture (250 µl bacterial culture with OD₆₀₀ of about 1 per 100 ml LB media). Four wells at the distance of approximately 3 cm were made by using 4 mm cork borer. Two wells were for sample, 50 µl of each dilution (50 and 100 mg/ml), was poured into each well. Two wells were loaded with controls, one with 50 µl gentamycin (40 mg/ml) as positive control and other with 50 µl of negative control, dimethyl sulfoxide (DMSO). The plate was then incubated at 37°C overnight. Diameter of zone of inhibition was measured in millimeters. This procedure is followed to all extracts like CAE, CME, CEE, CEAE, and CPEE. The experiment was replicated thrice.

Determination of minimum inhibitory concentration (MIC)

50 µl of bacterial culture (OD₆₀₀ = 1.0) was inoculated in each LB media tubes containing 10 to 150 mg/ml sample. Two negative

controls were employed, one was LB broth only and the second one was LB broth with extract (100 mg/ml). Positive control was LB broth and a test organism. After 24 h incubation at 37°C, absorbance of suspension was measured, using spectrophotometer at wavelength (λ) = 600 nm. The concentration of test sample at which growth of bacterial culture was inhibited was considered as MIC (Kumar et al., 2011; Etebong and Nwafor, 2009).

Determination of minimum bactericidal concentration (MBC)

Loop full of broth from each tube in MIC determination was streaked on LB agar plates and incubated at 37°C for 16 to 20 h. MBC was determined as the concentration of test sample at which no bacterial growth was seen (Kumar et al., 2011).

RESULTS

Percentage yield

The percentage yield for different solvents used to prepare extract from peels of *R. sativus*. Highest yield was of CEE (5.6%) followed by 5% of CPEE, 4.2% of CME, 3.5% of CEAE and lowest was of aqueous extract (3.3%).

Phytochemical analysis

Phytochemical analysis revealed the presence of tannins, saponins, flavonoids, phlobatannins, anthraquinones, carbohydrates, reducing sugars, steroids, phytosterol, alkaloids, amino acids, terpenoids, cardiac glycosides and chalcones in *R. sativus niger* extracts (Table 1).

Proximate analysis

Seven percent (7%) moisture content is present in the

Table 2. Zone of inhibition in mm of 100 and 50 mg/ml dilutions of different extracts of *Raphanus sativus niger*.

Bacterial strains	Zone of inhibition in mm											
	CAE		CME		CEE		CEAE		CPEE		Gentamycin	
	100 (mg/ml)	50 (mg/ml)	100 (mg/ml)	50 (mg/ml)	100 (mg/ml)	50 (mg/ml)	100 (mg/ml)	50 (mg/ml)	100 (mg/ml)	50 (mg/ml)	100 (mg/ml)	50 (mg/ml)
Gram positive												
<i>S. aureus</i>	22 ± 2.2	18 ± 1.5	18 ± 2.1	3 ± 2.6	25 ± 0.3	20 ± 0.6	26 ± 0.7	23 ± 1.2	25 ± 1.5	21 ± 1.8	24 ± 0.8	21 ± 1.4
<i>B. subtilis</i>	24 ± 0.4	21 ± 0.6	25 ± 1.2	23 ± 0.7	27 ± 0.8	21 ± 1.0	31 ± 0.6	26 ± 0.7	29 ± 1.2	26 ± 1.4	20 ± 1.2	20 ± 1.1
<i>M. lutes</i>	18 ± 0.03	17 ± 0.6	18 ± 2.1	17 ± 1.8	28 ± 0.9	23 ± 1.5	25 ± 1.3	21 ± 1.1	21 ± 0.7	19 ± 1.0	25 ± 1.4	21 ± 2.1
<i>E. aerogenes</i>	18 ± 1.1	16 ± 1.9	23 ± 0.9	20 ± 1.3	24 ± 1.5	19 ± 1.0	23 ± 2.1	22 ± 2.7	21 ± 1.7	16 ± 1.2	20 ± 1.9	20 ± 0.9
<i>S. typhi</i>	16 ± 1.6	8 ± 1.4	12 ± 1.4	5 ± 1.6	11 ± 1.1	3 ± 1.3	17 ± 0.9	13 ± 1.5	12 ± 1.1	3 ± 1.2	23 ± 1.8	21 ± 2.3
Gram negative												
<i>E. coli</i>	15 ± 1.5	13 ± 1.4	25 ± 1.6	24 ± 1.3	24 ± 1.0	21 ± 1.1	26 ± 0.9	24 ± 0.4	28 ± 1.2	25 ± 1.6	33 ± 1.1	32 ± 0.8
<i>K. pneumoniae</i>	20 ± 2.1	17 ± 1.8	19 ± 1.6	19 ± 1.0	21 ± 1.6	17 ± 2.4	25 ± 1.7	19 ± 1.4	23 ± 0.2	18 ± 1.5	14 ± 1.5	11 ± 1.0
<i>P. aeruginosa</i>	11 ± 1.3	6 ± 1.9	18 ± 1.6	11 ± 1.1	19 ± 0.6	14 ± 0.8	18 ± 3.5	12 ± 0.8	21 ± 1.7	15 ± 1.5	31 ± 1.2	25 ± 0.9
<i>B. bronchiseptica</i>	15 ± 0.9	14 ± 0.8	21 ± 1.1	18 ± 0.9	24 ± 0.8	22 ± 0.7	21 ± 1.4	13 ± 1.9	19 ± 1.6	18 ± 0.7	26 ± 1.4	21 ± 1.1

sample. 93% dry matter peels of *R. sativus niger* was composed of crude protein (28.57%), fats (27.76 %) and carbohydrates (39.82 %), while fibers were only 1.4% and ash content was around 2.43%.

Antibacterial activity

Zone of inhibition in mm is given in Table 2. Extract concentration of 100 mg/ml is effective against both gram positive and gram negative bacterial strains tested. Ethyl acetate extract was most effective against *S. aureus*, *B. subtilis*, *S. typhi* and *K. pneumoniae*. Ethanol extract had highest zone of inhibition against *M. luteus*, *P. aeruginosa*, *B. bronchiseptica* and *E. aerogenes*. Zone of inhibition for petroleum ether extract is 28 ± 1.2 mm, highest compare to other four extracts (aqueous, methanolic, ethanolic and ethyl

acetate), against *E. coli*. From the average of cumulative zone of inhibition (Figure 1), it is deduced that as a whole 100 and 50 mg/ml of aqueous extract (averages = 18 ± 1.2 and 14 ± 0.9 mm, respectively) were least effective while the dilutions: 100 and 50 mg/ml of ethyl acetate (averages = 24 ± 1.4 and 19 ± 1.1 mm, respectively) showed pronounced inhibition against bacterial strains used in the study.

Minimum inhibitory concentration (MIC)

Ethanolic and ethyl acetate extracts were most effective of all extracts, so MIC value of only these two extracts was determined. Figure 2 indicates the MIC values of ethanolic and ethyl acetate extract against all the bacterial strains tested. Lowest MIC value (30 mg/ml) was of ethyl acetate extract against *E. coli*, while highest value (70

mg/ml) was against *Enterobacter aerogenes* gram positive and gram negative bacteria. Ethanolic extract had lowest MIC value, that is, 40 mg/ml against *S. aureus*, *E. coli* and *B. bronchiseptica*; its highest MIC value was against *S. typhi* and *K. pneumoniae*.

Minimum bactericidal concentration (MBC)

According to values of MBC (Figure 3), both ethanol and ethyl acetate extracts were equally active against *E. coli*, having MBC = 50 mg/ml. Ethyl acetate was least effective against *S. typhi* with highest MBC value of 120 mg/ml.

DISCUSSION

The members of family Brassicaceae are rich in

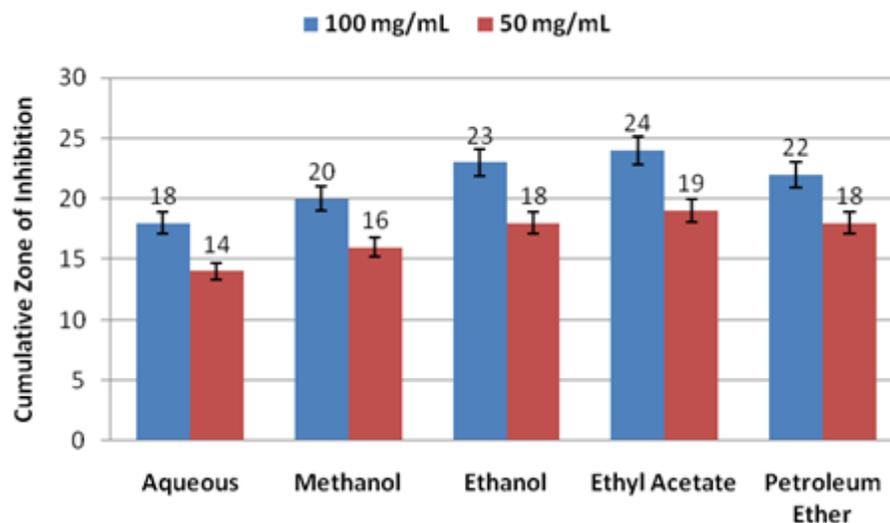


Figure 1. Average of cumulative zone of inhibition (mm) of each extract against all bacterial strains tested.

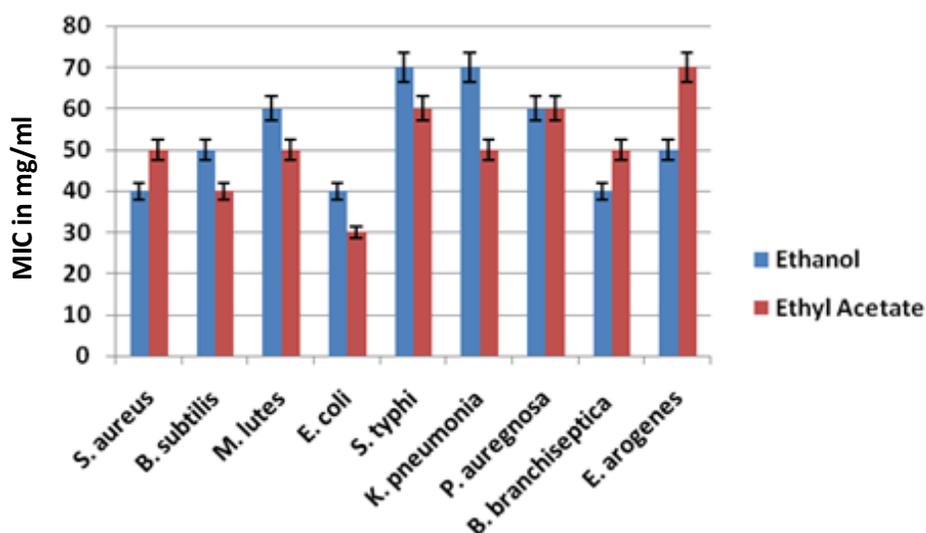


Figure 2. Minimum inhibitory concentration (MIC) values of ethanolic and ethyl acetate extract against gram positive and gram negative bacteria.

phytochemicals (Esaki and Onozaki, 1982; Uda et al., 1993; Nakamura et al., 2008; Beevi et al., 2009; Bjorkman and Shail, 2010), and have potential medicinal roles including antimicrobial, antifungal, antimutagenic, antioxidant and antitumor (Ghazanfar and Al-Al-Sabahi, 1993). *R. sativus niger*, one of the member of the family Brassicaceae, is rich in many important chemical constituents (Rani et al., 2008; Kim et al., 2011; El-Tohamy et al., 2010). After the reports about medicinal attributes of the *R. sativus* leaves (Kim et al., 2011), roots (Hanlon and Barnes, 2010) and seeds (Rani et al., 2008; El-Tohamy et al., 2010), root peels which are waste material were tested, particularly for phytochemicals and

antimicrobial potentials. The phytochemical analysis of different varieties of *R. sativus* elucidated the presence phytochemicals which are active antibacterial agents, like glucosinolates, isothiocyanates and phenolic compounds like anthocyanins and anthocyanidins (Friis and Kjar, 1966; Papi et al., 2008; Rani et al., 2008; Valgimigli and lori, 2009; Hanlon and Barnes, 2010). Table 1 depicts the phytochemical profile of peels of *R. sativus*, most of the constituents which are present in leaves, roots and seeds of *R. sativus*. Results of proximate analysis are supported by the report of El-Tohamy et al. (2010), that *R. sativus niger* roots have proteins, carbohydrates, lipids and fibers in considerable amount.

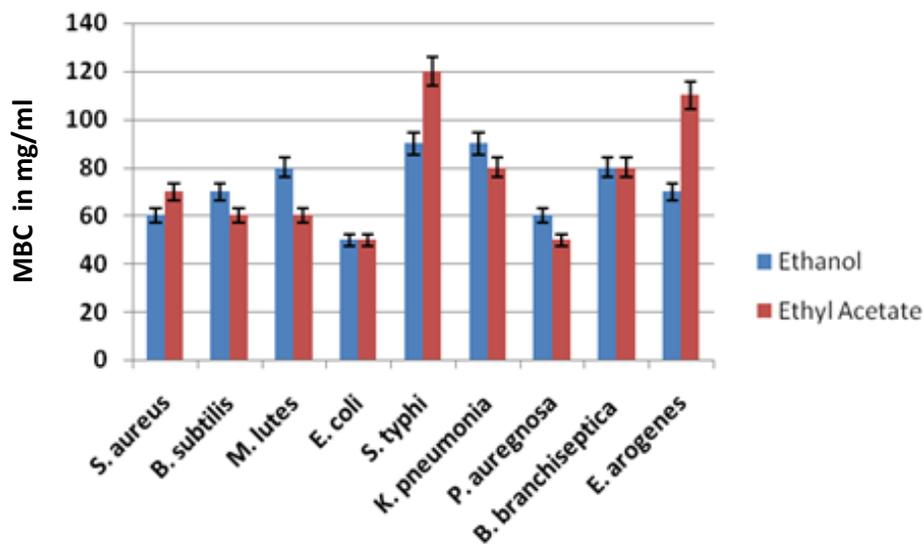


Figure 3. Minimum bactericidal concentration (MBC) values of ethanolic and ethyl acetate extract against gram positive and gram negative bacteria.

For determination of medicinal value of *R. sativus* numerous studies used different types of solvents for extraction of biologically active constituents of *R. sativus* like: aqueous (Ghayur and Gilani 2005; Lugasi et al., 2005; Hanlon et al., 2009), organic solvents for instance, methanolic (Takaya et al., 2003; Salah-Abbes et al., 2009) and ethanolic (Kim et al., 2011), and hydrophobic solvents viz chloroform and toluene (Yamasaki et al., 2009; Beevi et al., 2009). These extracts exhibited different biological activities (Chevallier, 1996; Gutierrez and Perez 2004; Lugasi et al., 2005; Shukla et al., 2010; Wang et al., 2010; Kim et al., 2011; Nakamura et al., 2001; Papi et al., 2008; Yamasaki et al., 2009; Beevi et al., 2009; Bown, 1995). The reported fact is that *R. sativus* niger roots, leaves and seeds have antimicrobial agents (Rani et al., 2008; Hanlon and Barnes, 2010; Kim et al., 2011) and different extracts prepared from the peels of this edible root also exhibited antibacterial activity (Table 2); indicating its pharmaceutical potential for development of new alternative medicine.

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